

JOURNAL
OF
SOUTH AFRICAN BOTANY
VOL. XIV.

OBSERVATIONS ON SOME WATER FUNGI
COLLECTED IN AND AROUND JOHANNESBURG

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(With Plates XV—XVII.)

I. INTRODUCTION.

After a period of relative inactivity, research on aquatic Phycomycetes has shown, during the past 20 years, a remarkable revival. Sparrow (1935) traced this fresh impetus not only to the success of a few stimulating teachers in transmitting to their students their own enthusiasm for these peculiar and obscure organisms, but also to the newly-awakened interest of mycologists and botanists in general in the significance of the lower fungi in interpreting biological phenomena and relationships in other more highly organised groups.

Research done during the past ten years, particularly on the Saprolegniaceae and *Pythium*, seemed as intensive and productive as that of the decade so ably reviewed by Sparrow (1935), and must, in fact, be regarded as a direct continuation of that phase. From all over the world have come descriptions of new species and records of new localities and new hosts for species already known. New taxonomic treatments for some of the genera have been put forward. Work done in Germany, Australia and Mexico, taken in conjunction with earlier investigations in the United States of America, England, Austria, Latvia and Japan, has indicated a distinct lack of endemism in the distribution of aquatic fungi in the soil. Ivimey-Cook & Morgan (1934) were so impressed by the wide distribution and large variety of Saprolegniaceae in the soil as to suggest that the common name "water moulds" for these fungi should

logically be given up. The cytology of a large number of species has been investigated by various workers. Pathogenic species of *Pythium* have received considerable attention, and it now seems fairly well established that the genus is consistently homothallic.

Raper (1935 *et. seq.*) showed that five hormones were involved in a mutually complementary manner in initiating and co-ordinating the different stages of sexual reaction between the male and female plants of *Achlya ambisexualis*. The same writer (1940) considered that the sexuality of the heterothallic members of the Saprolegniaceae could be explained on a scheme similar to that suggested by Hartmann (1929) and Vandendries (1930). Couch (1941) described dark field studies on the reaction of the cilia of living zoospores of *Saprolegnia ferax*.

Three papers on South African Phycomycetes have been published so far—du Plessis' paper on *Olpidiopsis ricciae* (1933) and Wager's paper on pathogenic species of *Pythium* and *Phytophthora* (1941). Investigating root rot diseases of vegetable and garden plants, the latter found that species of *Pythium* were fairly common and widely-distributed in this country, assigning his isolates to ten known series. Wolf (1941) reported that he isolated *Allomyces arbuscula* from soil samples collected near Balfour, Cape Province, by Miss E. S. Moore who sent them to him.

The present paper is concerned mainly with the identification and description of members of the Saprolegniaceae and species of *Pythium* isolated from eight pools at Frankenwald, the Botanical Research Station of the University of the Witwatersrand, together with records of their occurrence during one calendar year. Samples of water were also collected sporadically from the Zoo Lake and several mine dams along the Witwatersrand.

2. METHODS USED.

From November 1944, to November 1945, samples of water were collected from eight pools at Frankenwald. The samples were taken near the surface, and any twigs, dead insects, etc., seen floating around were included. In each case, at X, A and C two samples were taken, one near the surface and the other from the deepest accessible region. When the pools dried up, samples of mud were collected. Records were kept of the colour and turbidity of the water, presence of algae, change in water level and general appearance of the pools. The samples were brought to the laboratory as soon as possible, where they were uncorked.

The following day, the samples were transferred to shallow dishes and a small amount of tap-water was added. Several sterilised house flies and hemp seed were scattered over the surface to serve as baits. The dishes were kept away from direct sunlight and partly covered with

plate glass to exclude dust while allowing free access to the air. Samples from the Zoo Lake and the mine dams were treated in the same way. Mud samples were placed in petri dishes, tap water was added to a depth of about 1 cm. and baits were set out. The baits were inspected on the 2nd, 4th and 6th days after setting out and records were kept of the species found in each sample.

Pure cultures were obtained by successive transfers, taking advantage of any differences in growth rates and nutritional peculiarities. A bait with a young and vigorous mycelium of the fungus to be identified was selected, and, held with forceps, rotated in a gentle trickle of tap water and placed in a petri dish with fresh baits in sterilised tap water. For species of *Achlya* and *Aphanomyces* a single group of zoospores was drawn up into a pipette made of capillary tubing and placed with fresh baits. If sexual reproduction did not take place when the cultures were reasonably clean, the fungus was grown on a variety of media—including pea decoction, oat-meal agar, corn meal agar, malt agar, malt peptone agar, carrot slices, prune slices, split maize grains and ant pupae—to induce this phase. In general, flies, hemp seeds and maize grains gave the best results, and further details of success are given under the descriptions of the species found. Where the original description was unobtainable in South Africa, identification was based mainly on descriptions given in Coker's monograph on the Saprolegniaceae (1923) and Middleton's monograph on *Pythium* (1943).

3. DESCRIPTIONS OF THE SPECIES.

(1) *Saprolegnia delica* Coker.

Figs. 1, 2, 3. pl. XV.

DESCRIPTION.

Mycelium a dense growth of stout hyphae on house fly baits. *Hyphae* cylindrical, main hyphae sparingly branched. *Sporangia* subcylindrical, of slightly larger diameter than the hyphae on which they arise, primary sporangia terminal on the main hyphae, secondary sporangia formed by repeated proliferation from within the primary, symmetrically and characteristically constricted, rarely arising from below the primary. *Zoospores* discharged in the manner typical for the genus. *Gemmae* spherical, pyriform or clavate, often in chains. *Oogonia* smooth, terminal on short lateral branches or on the main hyphae, typically spherical, wall often with a few inconspicuous pits in addition to those under the antheridia. *Antheridia* typically several per oogonium, declinuous more often than monoclinal (*i.e.* obviously arising from the same mycelium

as the oogonium to which it is applied), antheridial cells bulging irregularly, applied along the length to the oogonial wall, antheridial branches of various lengths, frequently long and rambling, the declinuous ones usually arising from hyphae which also bear oogonial branches, fertilisation tubes formed. *Oospores* 1—14, mostly 4—10, in an oogonium, wall thick (*i.e.* more than $1.8\ \mu$ thick), a single reserve globule enclosed in the protoplasm.

Measurements.		Range	Average
		in μ .	in μ .
Diameter of hyphae		23—42	34
Length of sporangia		88—243	140
Diameter of zoospores		10—12	10.5
Diameter of oogonia		18—123	64
Diameter of oospores		22—29	25

NOTES.

1. This species was isolated from water from Frankenwald, the Zoo Lake and Spaarwater dam and from damp mud from Frankenwald. It was originally described from the United States of America (Coker, 1923) and has been recorded for Europe but not yet for South Africa.

2. The secondary sporangia were symmetrically and gracefully constricted, and proliferation tended to give the effect of a nest of distinctively-shaped tiny vases.

3. The fatty reserve in the oospore invariably took the form of a single large drop which stained the characteristic bright orange colour with Sudan III. This would appear to be most unusual for a species of *Saprolegnia* as, according to Coker (1923), the oospore, throughout the genus, has one or two layers of small fat droplets entirely surrounding the protoplasm.

4. Antheridia and oogonia were readily produced on house fly.

(2) *Achlya imperfecta* Coker.

Figs. 4, 5. pl. XV.

DESCRIPTION.

Mycelium a dense growth of stout threads on house fly and hemp seed baits. *Hyphae* tapering gradually towards the tips, main hyphae sparingly branched. *Sporangia* subcylindrical, of slightly larger diameter than the hyphae on which they arise, primary sporangia terminal on the main hyphae, secondary sporangia formed in a closely clustered sympodium by lateral branching from below the primary. *Zoospores* discharged

in the manner typical for the genus. *Gemmae* formed by segmentation of the hyphae behind the sporangia, subcylindrical and in rows. *Oogonia* smooth, terminal on short lateral branches, spherical, wall without pits except under the antheridia, rarely with 1 or 2 inconspicuous ones. *Antheridia* typically several per oogonium, monoclinal more often than declinal, antheridial cells bulging irregularly, applied along the length to the oogonial wall, antheridial branches of various lengths, frequently long and rambling, most commonly arising from hyphae which also bear oogonial branches, sometimes from hyphae which give rise to antheridial branches only, rarely from the oogonial stalks, fertilisation tubes not seen. *Oospores* 3—10, mostly 4 or 5, in an oogonium, often ellipsoidal due to pressure, wall thick, a single reserve globule outside the protoplasm, sometimes disorganising before maturity.

Measurements.	Range in μ .	Average in μ .
Diameter of hyphae	28 — 111	48
Length of sporangia	240 — 502	278
Diameter of zoospores	9 — 12	11
Diameter of oogonia	32.5 — 47	43
Diameter of oospores	18 — 23	20

NOTES.

1. This species was isolated from water from Frankenwald, the Zoo Lake and Spaarwater dam and from damp mud from Frankenwald. It was originally described from the United States of America (Coker, 1923) and has also been recorded for Europe and Japan, but not yet for South Africa.

2. In some cases the reserve globule in the oospore degenerated and in others the oospore as a whole degenerated at an early stage. This was taken as corresponding to Coker's "early dissolution" and "going to pieces before maturity". The majority of the oospores, however, matured normally as did the oospores in *Achlya deBaryana* var. *intermedia* Minden which Coker considered identical with his *A. imperfecta*.

3. Antheridia and oogonia were produced fairly readily on house fly and hemp seed.

4. This species developed best during hot weather.

(3) ***Achlya dubia*** Coker.

Figs. 6, 7, pl. XV, 8. pl. XVI.

DESCRIPTION.

Mycelium a dense growth of stout threads on house fly baits. *Hyphae* tapering gradually towards the tips, main hyphae sparingly branched. *Sporangia* subcylindrical, of slightly larger diameter than the hyphae on which they arise, primary sporangia terminal on main hyphae, secondary sporangia formed sparingly by lateral branching from below the primary. *Zoospores* at first discharged as in *Thraustotheca*, a large mass of protoplasm at the tip of the sporangium sometimes remaining undivided, later as in *Achlya* and occasionally as in *Dictyuchus*. *Gemmae* formed by segmentation of the hyphae behind the sporangia, subcylindrical and in rows. *Oogonia* smooth, terminal on short lateral branches, rarely terminal on the main hyphae, spherical, wall without pits except under the antheridia. *Antheridia* typically several per oogonium, declinous, antheridial cells applied along the length to the oogonial wall, usually cylindrical and partly encircling the oogonium, antheridial branches of various lengths, most often arising as branches of the main hyphae, sometimes terminating the main hyphae, fertilisation tubes formed. *Oospores* 2—6, frequently 3, in an oogonium, wall thick, a single reserve globule outside the protoplasm.

Measurements.		Range	Average
		in μ .	in μ .
Diameter of hyphae		27—93.5	36
Length of sporangia		97—336	180
Diameter of zoospores		7—12.5	11
Diameter of oogonia		39—195	61
Diameter of oospores		18—27	20.5

NOTES.

1. This species was isolated from both damp and dry mud from Frankenswald. It was originally described from the United States of America (Coker, 1923), and has been recorded for Europe but not yet for South Africa.

2. Antheridia and oogonia were produced fairly readily on house fly.

(4) *Dictyuchus monosporus* Leitgeb.

Figs. 9, 10. pl. XVI.

DESCRIPTION.

Mycelium a dense growth of stout hyphae on house fly baits and hemp seed baits. *Hyphae* tapering gradually towards the tips, main hyphae sparingly branched. *Sporangia* subcylindrical or clavate, of slightly larger diameter than the hyphae on which they arise, primary sporangia

terminal on the main hyphae, secondary sporangia at first formed in an elongated sympodium, later by segmentation of the hyphae, frequently becoming detached from the hyphae and going into a resting condition in which the vacuoles of the zoospores become conspicuous. *Zoospores* discharged in the manner typical for the genus and leaving a network of persistent walls. *Oogonia* smooth, terminal on lateral branches of various lengths, spherical or subspherical, wall without pits. *Antheridia* 1 or 2 per oogonium, declinuous, antheridial cells applied along the length to the oogonial wall, typically cylindrical and partly encircling the oogonium, antheridial branches of various lengths, fertilisation tubes not seen. *Oospores* solitary.

Measurements.			Range	Average
			in μ .	in μ .
Diameter of hyphae ..	..	..	15—80	45
Length of sporangia ..	..	..	180—789	320
Diameter of zoospores ..	..	..	7— 14·5	10·5
Diameter of oogonia ..	..	..	32— 43	35

NOTES.

1. This species was isolated from water from Frankenwald and Spaarwater Dam and from damp mud from Frankenwald. It was originally described from Europe (Leitgeb, 1869) and also has been recorded for the United States of America and Australia, but not yet for South Africa.

2. Antheridia and oogonia were produced rarely on fly, probably because the male and female strains were present together only on rare occasions.

3. No mature oospores were seen.

4. This species was of somewhat tardy development on the baits.

(5) *Aphanomyces laevis* de Bary.

Figs. 11, 12, pl. XVI.

DESCRIPTION.

Mycelium a dense growth of delicate threads on house fly baits. *Hyphae* cylindrical, sparingly branched. *Sporangia* not differentiated from vegetative hyphae, sometimes sparingly branched, often arising from the substratum. *Zoospores* discharged in the manner typical for the genus. *Oogonia* smooth, terminal typically on short lateral branches, spherical or subspherical, wall without pits. *Antheridia* typically several per oogonium, monoclinal or declinuous, antheridial cells bulging irregularly, applied along the length to the oogonial wall, often elongated and wrap-

ping spirally round the oogonium, antheridial branches of various lengths, usually coiling round the oogonial branches in a complicated way, fertilisation tubes formed. *Oospores* solitary, not filling the oogonial cavity, wall thick, a single reserve globule enclosed in the protoplasm.

Measurements.		Range	Average
		in μ .	in μ .
Diameter of hyphae ..	..	4.5—8	6
Diameter of zoospores ..	..	9 —12.5	10
Diameter of oogonia ..	..	24 —32	28
Diameter of oospores ..	..	17 —25	20

NOTES.

1. This species was isolated from water from Frankenwald. It was originally described from Europe (de Bary, 1860) and has also been recorded for the United States of America, but not yet for South Africa.

2. Petersen (1910), Coker (1923) and Couch (1926) all pointed out that this was probably a very variable species, and it was Coker's opinion that the limits of *A. laevis* might be extended to include *A. helicoides* Minden. Cutter (1941), however, considered *A. helicoides* specifically distinct, basing his separation of the two species on the thickness of the oospore wall. Minden (1915) neither mentioned the thickness of the oospore wall nor gave any drawings; but in Cutter's material the oospore wall never reached a thickness greater than 1.5μ . Several investigators have reported that the oospore wall in *A. laevis* measured up to 3μ in thickness. In the Frankenwald fungus the oospore wall attained the latter thickness and, for this reason, according to Cutter, it should be determined as *A. laevis* in spite of the pronounced winding of the antheridial branches. Further points of difference from Minden's fungus were the indiscriminate occurrence of oogonia throughout the mycelium and the absence of any clusters of short intertwined branches forming characteristic knots that might correspond to Minden's "antheridial tangles".

3. Antheridia and oogonia were rarely and apparently capriciously produced on house fly.

4. As Peters (1911) had isolated this species from sugar beets affected by root rot, attempts were made to infect beet and "mangold wurzel" seedlings. These were not successful. Smith (1940) also failed to obtain infection.

(6) ***Aphanomyces stellatus*** de Bary.

Fig. 13. pl. XVI.

DESCRIPTION.

Mycelium a dense growth of delicate threads on house fly baits. *Hyphae* cylindrical, sparingly branched. *Sporangia* not differentiated from vegetative hyphae, sometimes sparingly branched, often arising from the substratum. *Zoospores* discharged in the manner typical for the genus. *Oogonia* smooth when young, later developing numerous blunt papillae, terminal typically on short lateral branches, subspherical, wall without pits. *Antheridia* typically several per oogonium, monoclinal or dichinous, antheridial cells bulging irregularly, applied along the length to the oogonial wall, antheridial branches of various lengths, fertilisation tubes formed. *Oospores* solitary, not filling the oogonial cavity, wall thin (*i.e.* less than 1.8μ thick), numerous reserve droplets in an inconspicuous crescent to one side of the protoplasm.

Measurements.				Range	Average
				in μ .	in μ .
Diameter of hyphae	..	..		3.5—7.5	6
Diameter of zoospores	..	..		7 —10	9
Diameter of oogonia (including papillae)	..	..	..	18 —36	25
Diameter of oospores	..	..		14.5—21.5	20

NOTES.

1. This species was isolated from water from Frankenwald. It was originally described from Europe (de Bary, 1860) and has also been recorded for the United States of America and Japan, but not yet for South Africa.

2. Antheridia and oogonia were rarely and apparently capriciously produced on house fly.

(7) **Pythium dissotocum** Dreschler.

Figs. 14, 15, 16. pl. XVII.

DESCRIPTION.

Mycelium a dense growth of delicate threads on house fly and hemp seed baits. *Hyphae* cylindrical, with short, somewhat swollen dactyloid outgrowths, sparingly branched. *Sporangium* not differentiated from vegetative hyphae, sometimes sparingly branched or including short, somewhat swollen dactyloid outgrowths. *Zoospores* discharged in the manner typical for the genus, 15—24 or more in a vesicle. *Oogonia* smooth, typically terminal, sometimes intercalary, spherical or sub-

spherical. *Antheridia* 1 or 2 per oogonium, monoclinal more often than declinal, antheridial cells crook-shaped in outline, making broad apical contact with the oogonial wall, usually borne on a relatively short branch, occasionally sessile, fertilisation tubes formed. *Oospores* solitary, smooth, almost filling the oogonial cavity, wall thick, a single reserve globule and refringent body enclosed in the protoplasm.

Measurements.			Range	Average
			in μ .	in μ .
Diameter of hyphae	..	..	3.5—7	4
Diameter of zoospores	..	..	7 — 9	8.5
Diameter of oogonia	..	..	16 —36	21
Diameter of oospores	..	..	12.5—32.5	19

NOTES.

1. This species was isolated from water from Frankenwald, the Zoo Lake, Spaarwater Dam and United Roodepoort Dam, and from damp and dry mud from Frankenwald. It was originally described from the United States of America (Dreschler, 1930) and has also been recorded for Japan, but not yet for South Africa.

2. *Antheridia* were rarely and apparently capriciously produced on hemp seed.

3. Some of the strains grew only on house fly and others only on hemp seed, thus indicating a physiological differentiation within the species.

(8) *Pythium perniciosum* Serbinow.

Figs. 17, 18. pl. XVII.

DESCRIPTION.

Mycelium sparse and delicate on hemp seed baits. *Hyphae* cylindrical, frequently swelling out into irregular bulging regions or into catenulate, spherical asexual reproductive bodies of which there are 2—6 in a chain, sparingly branched. *Sporangia* not differentiated from vegetative hyphae, sometimes arising from irregular swollen regions of the hyphae. *Zoospores* discharged in the manner typical for the genus, 15—24 in a vesicle. *Oogonia* smooth, terminal or intercalary, subspherical. *Antheridia* usually 1, occasionally 2 or 3 per oogonium, monoclinal or declinal, antheridial cells slightly curved, making narrow apical contact with the oogonial wall, antheridial branches of various lengths, fertilisation tubes formed. *Oospores* solitary, rarely 2 in an oogonium, smooth, not filling the oogonial cavity, wall thick, a single reserve globule and refringent body enclosed in the protoplasm.

Measurements.	Range in μ .	Average in μ .
Diameter of hyphae	3.5—7	4
Diameter of asexual reproductive bodies	14.5—29	27
Diameter of zoospores	7—11	9
Diameter of oogonia	31.5—36	31
Diameter of oospores	14.5—29	20

NOTES.

1. This species was isolated from water from United Roodepoort Dam. It was originally described from Russia (Serbinow, 1912) and has also been recorded for the United States of America, Europe, the Netherland East Indies, but not yet for South Africa.

2. Antheridia and oogonia were produced fairly readily on hemp seed.

3. As Middleton (1943) had isolated this species from rotted Poinsettia cuttings, attempts were made to infect Poinsettia plants. These were not successful, but the fungus was grown in water cultures on pieces of Poinsettia stem.

(9) **Pythium polytylum** Dreschler.

Figs. 19, 20, 21. pl. XVII.

DESCRIPTION.

Mycelium a dense growth of delicate threads on house fly and hemp seed baits. *Hyphae* cylindrical, sparingly branched. *Sporangia* spherical or subspherical, resting sporangia papillate, arising terminally though sometimes assuming a lateral position due to resumption of growth by the supporting hypha, proliferous. *Zoospores* discharged in the manner typical for the genus, 9—23 in a vesicle. *Oogonia* smooth, typically terminal, sometimes intercalary or lateral, subspherical, often protruding irregularly towards the antheridia. *Antheridia* 1—5, mostly 1—3, per oogonium, typically declinous, occasionally monoclinalous, antheridial cells often elongated and irregularly wavy in outline, applied along the length to the oogonial wall, sessile or borne on branches of various lengths, on a comparatively long branch when monoclinalous, fertilisation tube arising from the centre of the region applied to the oogonium. *Oospores* solitary, smooth, not filling the oogonial cavity, wall thick, 3—12 reserve globules and several refringent bodies enclosed in the protoplasm.

Measurements.		Range in μ .	Average in μ .
Diameter of hyphae		2 — 7	4
Diameter of sporangia		21.5—30	28
Diameter of zoospores		8 —10	9
Diameter of oogonia		30 —43	34
Diameter of oospores		21.5—32.5	28

NOTES.

1. This species was isolated from water from Frankenwald, the Zoo Lake and Spaarwater Dam, and from damp mud from Frankenwald. It was originally described from the United States of America (Dreschler, 1930) and has not been recorded for any other country.

2. Antheridia and oogonia were produced fairly readily on split maize grain.

(10) *Pythium ultimum* Trow.

Fig. 22. pl. XVII.

DESCRIPTION.

Mycelium a dense growth of delicate threads on house fly and hemp seed baits. *Hyphae* cylindrical, much branched. *Sporangia* spherical or subspherical, terminal or lateral, rarely intercalary, germinating by germ tubes or developing a single conspicuous vacuole, enlarging and assuming irregular shapes. *Oogonia* smooth, terminal, rarely intercalary, spherical. *Antheridia* typically 1 per oogonium and monoclinalous from just next to it, occasionally 2 in which case one may be declinalous, antheridial cells sometimes not delimited by a septum, when monoclinalous sharply curved to make narrow apical contact with an adjacent part of the oogonial wall, when declinalous straight, antheridial branches short, fertilisation tubes formed. *Oospores* solitary, smooth, not filling the oogonial cavity, wall thick.

Measurements.		Range in μ .	Average in μ .
Diameter of hyphae		2 — 7	4
Diameter of sporangia		14 —32	24
Diameter of oogonia		21.5—25	23
Diameter of oospores		18 —23	19.5

NOTES.

1. This species was isolated from dry mud at Frankenwald. It was originally described from Europe (Trow, 1901) and has been recorded for the United States of America, Canada, the Philippine Islands, Australia,

New Zealand, Rhodesia and South Africa. It was described and figured for South Africa by Wager (1941) who isolated it from a large number of hosts and investigated its temperature growth relations.

2. Antheridia and oogonia were produced fairly readily on split maize grain.

3. No reserve globules were seen in the oospores.

(11) **Allomyces** sp.

Figs. 23, 24, 25. pl. XVII.

DESCRIPTION.

Mycelium a dense growth of stout threads on house fly and hemp seed baits. *Hyphae* tapering gradually towards the tips, divided into segments by pseudosepta which are perforated in the early stages, sparingly branched. *Sporangia* oval, typically in short chains at the ends of the hyphae, also solitary and arising terminally though often assuming a lateral position due to resumption of growth by the supporting hypha opening by 1 or 2 exit pores. *Zoospores* discharged singly. *Resting bodies* resembling the sporangia in shape and intermingled indiscriminately with them, wall thick and pitted, eventually lying free in the sheath formed by the cell wall and slipping out. *Gametangia* not observed.

Measurements.		Range	Average
		in μ .	in μ .
Diameter of hyphae		11— 38	17
Length between pseudosepta		20—402	210
Length of sporangia		40— 58	48
Diameter of sporangia		24— 36	26
Diameter of zoospores		7— 11	9.5
Length of resting bodies		36— 52	46
Diameter of resting bodies		25— 34	28

NOTES.

1. This species was isolated from damp and dry mud from Frankenburg.

4. SEASONAL DISTRIBUTION.

As a contribution to our knowledge of the seasonal distribution of the species found, the frequency of their occurrence was worked out on a percentage basis (see Table I, p. 149). A single record is given for both species of *Aphanomyces*, as it was difficult to distinguish between them on account of the similarity of the sporangia and of the rare and apparently

capricious production of antheridia and oogonia. Although no definite conclusions can be drawn from the results, they give some indication of the relative abundance with which the species tended to occur at different seasons.

The largest number of different species was found in the spring, during a severe drought. *Achlya dubia*, *Pythium ultimum* and *Allomyces* sp. were isolated only from mud samples collected in the spring. Without further investigation, it would be impossible to decide whether the appearance of these forms was influenced by the season or the weather, or whether their habitat was restricted to the mud at the bottom of the pools and did not extend to the water. There was a marked decline in the abundance of *Saprolegnia delica*, *Pythium dissotocum* and *Pythium polytylum* in the summer. *Dictyuchus monosporus*, on the other hand, was not found at all during the winter. *Aphanomyces* spp. decreased in abundance in the winter and a further decrease took place in the spring, which might be taken to indicate an adverse effect of drought on these species. *Pythium* sp. made its appearance only after the intense cold of the winter. *Achlya imperfecta* was present in about the same percentage of samples in all four seasons but, as previously noted, it developed best during hot weather.

Saprolegnia delica was the most abundant species in all the samples, both occurring with the greatest frequency and producing the largest number of mycelia. *Pythium dissotocum* and *Pythium polytylum* were clearly equal in abundance to some of the Saprolegniaceae.

5. WEATHER FOR THE YEAR.

At times, during the period of collecting, the weather reached extremes for our climate. December was exceptionally dry and the rainfall for March was unusually high. There was snow in May and extremely low temperatures were reached in June. Very little rain fell in the spring.

6. THE POOLS AT FRANKENWALD.

A preliminary inspection of the water-holes and water-courses at Frankenwald was undertaken and 8 pools, which seemed representative of the area, were chosen to collect from. Underlying the whole region is the old granite of the Witwatersrand system in which quartz crush zones and diabase dykes are common. The presence of the pools is due to the occurrence of either of the latter geological formations—Pools X, L, A, and R to quartz crush zones and B, C and N to diabase dykes, which in the case of B and C cross under the Jukskei River. The Jukskei arises within the municipal area of Johannesburg and makes its way to

TABLE I.
SEASONAL DISTRIBUTION.

Species.	November.	December.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	Summer.	Autumn.	Winter.	Spring.	All Samples.
<i>Saprolegnia delica</i> ..	53	52	44	50	77	62	86	82	81	95	72	67	49	74	85	69	66.4
<i>Achlya imperfecta</i> ..	58	40	18	45	35	38	27	18	24	45	39	33	37	34	35	36	34.7
<i>Achlya dubia</i> ..	—	—	—	—	—	—	—	—	—	—	6	6	—	—	—	6	0.8
<i>Dictyuchus monosporus</i>	—	8	6	32	19	8	—	—	—	—	6	33	11	9	—	19	9.4
<i>Aphanomyces</i> (2 species)	79	72	73	91	58	69	55	18	52	20	22	28	78	61	32	25	56.8
<i>Pythium dissotocum</i> ..	—	16	29	36	50	62	41	36	57	60	44	39	22	51	53	42	39.3
<i>Pythium perniciosum</i> ..	—	4	—	—	—	—	—	—	—	—	—	—	1	—	—	—	0.4
<i>Pythium polytylum</i> ..	—	4	18	41	38	58	45	18	33	50	33	39	16	47	36	36	31.7
<i>Pythium ultimum</i> ..	—	—	—	—	—	—	—	—	—	—	6	11	—	—	—	8	1.1
Water mould with resting bodies ..	—	—	—	—	4	—	—	—	—	—	—	—	—	1	—	—	0.4
<i>Pythium</i> sp. ..	—	—	—	—	—	—	5	—	10	10	11	6	—	1	8	8	3.1
<i>Pythium</i> sp. ..	—	—	—	—	—	—	—	—	—	—	—	6	—	—	—	6	0.8
<i>Allomyces</i> sp. ..	—	—	—	—	—	—	—	—	—	—	6	6	—	—	—	6	0.8
Number of species ..	19	25	34	22	26	26	22	11	21	20	18	18	100	74	52	36	262

Observations on Some Water Fungi Collected In and
Around Johannesburg.

Alexandra, a native township, and then through Frankenwald. B, L, N, R and S were never more than 2 ft. deep and X, A and C were usually 2—3 ft. deep. Except for some sedges at C, very little decaying material was found floating on the surface of the water. S was the only pool in which any movement was discernable. Apart from N, which was protected by a large overhanging willow, the pools were not in any way shaded. Glover (1937) described the species of flowering plants to be found round the pools and along the river. The surrounding soil is poor in terms of food values. Geese were kept at N. At times, cattle, sheep and donkeys had access to X, A, B, C and S, and the native farm labourers used the water of the same pools indiscriminately for all purposes.

Pool X.

The water was usually a dark, muddy brown and algae were rarely present. In the summer, there was a considerable drop in level but only during the spring drought did the pool dry up altogether, revealing rusty kitchen utensils, old boots and other rubbish in the mud. The western end of the pool was dammed-up with dead slangbos plants. The species of fungi found near the surface were the same as those found at a lower level, viz., *Saprolegnia delicata*, *Achlya imperfecta*, *Aphanomyces* sp., *Pythium dissotocum* and *Pythium polytylum*. *Achlya dubia*, *Pythium ultimum* and *Allomyces* sp. were isolated from the mud in the spring.

Pool A.

The water was usually an opaque, brownish grey and algae were occasionally present. The water level dropped considerably in the dry weather, but the pool did not dry up altogether during the time of collecting. For some weeks after the autumn rains, a considerable area surrounding the pool was marshy. The species of fungi found near the surface were the same as those found at a lower level, viz., *Saprolegnia delicata*, *Achlya imperfecta*, *Pythium dissotocum*, *Pythium polytylum*, and rarely *Dictyuchus monosporus*.

Pools B and C.

Although B and C are so close together, the water at C was often of a different colour and turbidity from that at B. At B it was always grey but at C it varied from bluish-grey to light brown, and at both pools the water varied from translucent to opaque. These differences may have been due to differences in the surrounding soil. Algae were usually present in both pools, frequently forming a fine scum at B and filamentous species growing attached to the decaying sedges on the surface at C. The water level at C varied far more than at B but neither of the pools dried up altogether during the time of collecting. The same species of fungi

were found at B and at C both near the surface and at a lower level, viz., *Saprolegnia delica*, *Achlya imperfecta*, *Dictyuchus monosporus*, *Aphanomyces stellatus*, *Pythium dissotocum* and *Pythium polytylum*.

Pool L.

The water was always clear and no algae were ever seen. The spring itself was walled off and covered over, and the pool formed by the water seeping through a crack in the wall and from which samples were taken dried up altogether several times during the summer and the spring. The fungi found were *Saprolegnia delica*, *Achlya imperfecta*, *Dictyuchus monosporus*, *Aphanomyces sp.*, *Pythium dissotocum* and *Pythium polytylum*. The strain of *Saprolegnia delica* in this pool was most prolific.

Pool N.

The water was usually a dark, brownish grey and varied from clear to opaque. Algae were often present. The water level dropped considerably in the dry weather, but the pool did not dry up altogether during the time of collecting. The fungi found were *Saprolegnia delica*, *Achlya imperfecta*, *Dictyuchus monosporus*, *Aphanomyces laevis*, *Pythium dissotocum* and *Pythium polytylum*.

Pool R.

The water was nearly always opaque and it varied a good deal in colour, changing from bluish-grey to brown and yellowish-green. Algae were sometimes present, forming a thick scum. The pool dried up altogether for several weeks during the summer and again for a shorter time during the spring. The fungi found were *Saprolegnia delica*, *Achlya imperfecta*, *Dictyuchus monosporus*, *Aphanomyces sp.*, *Pythium dissotocum* and *Pythium polytylum*. *Achlya dubia* was isolated from the mud in the spring.

Pool S.

Samples were taken from a quiet pool among the rocks along the east bank of the river. The water was usually clear, except after storms, when it was brown with silt. Algae were rarely present. There were only slight changes in the water level, and the pool did not dry up altogether during the time of collecting. The fungi found were *Saprolegnia delica*, *Achlya imperfecta*, *Dictyuchus monosporus*, *Aphanomyces sp.*, *Pythium dissotocum* and *Pythium polytylum*.

The results of a bacterial analysis of water samples taken after prolonged heavy rains are as follows :—

TABLE II.

Pools.		at 27°C.)													
		pH	Conductivity	In parts per 100,000	Total solids	Chlorine	Sulphuric oxide	Nitrogen as nitrate	Nitrogen as nitrite	Saline ammonia	Albuminoid ammonia	Oxygen absorbed (4 hours			
X.	A.	21	60	—	14.0	0.6	0.8	Trace	Nil	0.002	0.051	0.815	A.	during Drought.	0.425
		3.9	80	—	26.0	0.4	1.0	0.18	Trace	0.008	0.073	1.425			0.175
B.	B.	2.8	60	—	25.0	0.3	0.6	0.14	Trace	0.006	0.069	0.535	B.	during Drought.	0.440
		6.5	64	—	21.0	0.5	0.2	0.07	Trace	0.021	0.360	0.440			0.980
C.	C.	4.6	90	—	20.0	0.5	0.3	0.04	Trace	0.004	0.088	0.980	C.	during Drought.	0.035
		6.5	79	—	41.0	0.8	0.9	0.07	Trace	0.010	0.024	0.035			0.085
N.	N.	3.7	120	—	14.0	1.0	2.5	0.015	Trace	0.015	0.018	0.410	N.	R.	0.015
		3.1	160	—	18.0	0.8	2.2	0.003	Trace	0.003	0.043	0.410			0.475
S.	S.	10.6	600	—	61.0	6.4	10.8	0.081	Trace	0.081	0.043	0.475	S.	during Drought.	0.460
		8.7	590	—	60.0	10.9	5.6	0.12	Trace	0.10	0.012	0.460			0.066

Innumerable organisms per ml. growing at 37°C. *Bacillus coli* present in 1 ml., not isolated from 0.1 ml. in pools X, A and B.

Seventy-four organisms per ml. growing at 37°C. *B. coli* not isolated from 10 ml. in pool C.

Innumerable organisms present in pools L and N, per ml. growing at 37°C. *B. coli* present in 10 ml., not isolated from 1 ml.

Pool R.—Innumerable organisms present per ml. growing at 37°C. *B. coli* present in 1 ml., not isolated from 0.1 ml.

Pool S.—Innumerable organisms per ml. growing at 37°C. *B. coli* present in 0.1 ml.

The results of a chemical analysis of water samples are given in table II p. 152. Except where stated, the samples were taken after prolonged heavy rains.

7. DISCUSSION.

This investigation agrees with the findings of Maurizio (1896), that the water moulds are small fungi with an obvious mycelium able to live saprophytically. With only one exception, the samples as collected did not contain any mycelia but, in spite of this, whitish hyphae would soon appear on suitable baits.

To determine the genus of one of these fungi the discharge of zoospores must be observed, while to determine the species sexual reproduction must be observed, so that it is necessary to follow the complete life cycle for purposes of identification. This is sometimes a task for which time and patience are needed because, as Cotner (1930) showed, each species requires a certain definite set of conditions for the formation of typical zoospores, and many workers have found that the nutritional requirements for sexual reproduction are exacting and differ with each species. Although the fish parasite, *Saprolegnia parasitica*, has been studied more than any other member of the Saprolegniaceae, it was considered sterile for over 60 years. Kanouse (1932) inferred that there were physiological strains within the species, as some isolates reproduced sexually on a given medium while others did not, although identical stimuli were present. Numerous collections and cultures of *Dictyuchus monosporus* were made by Coker and his students; but only after 15 years did they obtain antheridia and oogonia, and then the genus was shown to be heterothallic (Couch, 1926). It was some years before Wager (1941) obtained any oogonia of *Pythium splendens*. Eventually, he discovered about a dozen in a three months' old culture, but the contents remained undifferentiated and oospores were not produced. Moreover, as is well-known, water moulds are very susceptible to changes of en-

vironment, and such changes may lead to variation even in those characters which have been regarded as constant and of value in classification. While the presence of forms intermediate between species and even between genera is, as Ivimey-Cook and Morgan (1934) indicate, of considerable interest from an evolutionary standpoint, it makes the taxonomic treatment correspondingly difficult.

The observations made in this investigation contribute no more than a mere fragment to our picture of the phycomycetous flora of this country. Little is known of the occurrence, periodicity and environmental factors influencing the growth of these fungi and, in all probability, many more species are to be found in and around Johannesburg. The recording for South Africa of the species described in this paper is of significance in emphasising the absence of endemism in their distribution.

Ordinarily, the presence of nitrogenous compounds such as nitrates, nitrites and ammonia in the pools would be regarded as evidence of organic pollution, but, in this instance their presence is more likely to be an indication of drainage of fertilisers from the pasture research plots. These substances could not have been without effect on the variety of the water moulds found and on their nutrition. The presence of *Bacillus coli* is evidence of organic contamination which would make the water unsafe for drinking. Certain fungi were isolated from all the pools at some stage during the time of collecting, viz., *Saprolegnia delica*, *Achlya imperfecta*, *Dictyuchus monosporus* (from all except X), *Aphanomyces* sp., *Pythium dissotocum* and *Pythium polytylum*. It is suggested that the strain of *Saprolegnia delica* at L might be suitable for the cytological investigation of sexual reproduction in this species as antheridia and oogonia were readily and abundantly produced, and that *Pythium* sp. might be suitable for studying the cytology of zoospore formation in this genus on account of the large size of the zoospores. Information on both these subjects, is, at present, lacking.

8. SUMMARY.

Fourteen species of water fungi were isolated and ten of them were identified as being already known species. Of the remaining forms the material obtained was not good enough for determination. Nine species were recorded, described and figured for South Africa for the first time, viz., *Saprolegnia delica*, *Achlya imperfecta*, *Achlya dubia*, *Dictyuchus monosporus*, *Aphanomyces laevis*, *Aphanomyces stellatus*, *Pythium dissotocum*, *Pythium perniciosum* and *Pythium polytylum*. A report of the occurrence of these species in this country emphasises the absence of endemism in their distribution. The seasonal distribution of the species found was worked out on a percentage basis for one calendar year. *Achlya*

dubia, *Pythium ultimum* and *Allomyces* sp. were isolated only from mud in the spring.

The species most commonly found at Frankenwald included *Saprolegnia delica*, *Achlya imperfecta*, *Dictyuchus monosporus*, *Aphanomyces laevis*, *Aphanomyces stellatus*, *Pythium dissotocum*, and *Pythium polytylum*. A chemical analysis of the water indicated that fertilisers from nearby pasture research plots were draining into the pools. A bacterial analysis showed that the water was unsafe for drinking.

9. ACKNOWLEDGMENTS.

I wish to thank Dr. J. Phillips, Professor of Botany at the University of the Witwatersrand, for allowing me the use of the facilities provided for research in his department ; Dr. S. Krupko for suggesting and guiding this investigation ; Mr. Britten, of the Government Chemical Laboratories, for doing the chemical analysis ; Dr. Lurie, of the South African Institute of Medical Research, for doing the bacterial analysis ; Dr. Meredith for many facilities offered during my work ; and Mrs. M. Moss for help in preparation of the work for publication.

10. BIBLIOGRAPHY.

1. BARY, A. DE : "Einige neue Saprolegnieen." Jahrb. f. wiss. Bot. 2 : 169. 1860.
2. COKER, W. C. : "The Saprolegniaceae, with notes on other Water Moulds." 1-201 Univ. of North Carolina Press. 1923.
3. COKER, W. C. and MATTHEWS, V. D. : "Saprolegniaceae." North American Flora 2 : 17-58. 1937.
4. COTNER, F. B. : "The Development of the Zoospores in the Oomycetes at Optimum Temperatures and the Cytology of their active Stages." Amer. Jour. Bot. 17 : 511-546. 1930.
5. COUCH, J. N. : "Notes on the genus Aphanomyces, with a description of a new Semiparasitic Species." Jour. Elisha Mitchell Soc. 41 : 213-226. 1926.
6. COUCH, J. N. : "Heterothallism in Dictyuchus, a Genus of Water Moulds." Ann. Bot. 40 : 849-881. 1926.
7. COUCH, J. N. : "The Structure and Action of the Cilia in Some Aquatic Phycomycetes." Amer. Jour. Bot. 28 : 704-713. 1941.
8. CUTTER, V. M., JR. : "Observations on certain Species of Aphanomyces." Mycologia 33 : 220-240. 1941.
9. DE PLESSIS, S. J. : "The Life History and Morphology of *Olpidiopsis ricciae* and Species infecting Riccia species in South Africa." Ann. Bot. 47 : 755. 1933.
10. DRESCHLER, C. : "Some New Species of Pythium." Jour. Wash. Acad. 20 : 398-418. 1930.
11. GLOVER, P. E. : "A Contribution to the Ecology of the Highveld Flora." S.A. Jour. Sci. 34 : 224-259. 1937.

12. HARTMANN, M.: "Verteilung, Bestimmung, und Vererbung des Geschlechts bei den Protisten und Thallophyten." In Baur & Hartmann Hundbuch der Vererbungswissenschaft 2E: 1-115. 1929.
13. IVIMEY-COOK, W. R. and MORGAN, E.: "Some Observations on the Saprolegniaceae of the Soils of Wales." Jour. Bot. 22: 345-349. 1934.
14. KANOUSE, B. B.: "A Physiological and Morphological Study of *Saprolegnia parasitica*." Mycologia 24: 431-452. 1932.
15. LEITGEB, H.: "Neue Saprolegnieen." Jahrb. f. wiss. Bot. 7: 375. 1869.
16. MAURIZIO, A.: "Die sporangium anlage der Gattung *Saprolegnia* Pringsh." Jahr. f. wiss. Bot. Bd. 29. 1896.
17. MIDDLETON, J. T.: "The Taxonomy, Host Range and Geographic Distribution of the Genus *Pythium*." Mem. Torrey Bot. Club 20: 1-171. 1943.
18. MINDEN, M.: "Pilze I. Phycomycetes." Krypt. fl. Brand. 5: 555-562. 1915.
19. PETERS, L.: "Ueber die Erreger des Wurzelbrandes." Arb. Biol. Anst. Land. forstn. 8: 211-259. 1911.
20. PETERSEN, H.: "An Account of Danish Fresh-water Phycomycetes." Ann. Myc. 8: 494-559. 1910.
21. RAPER, J. R.: "Sexual Hormones in *Achlya* I. Indicative Evidence for a Hormone Co-ordinating Mechanism." Amer. Jour. Bot. 26: 639-650. 1939.
22. RAPER, J. R.: "Sexuality in *Achlya ambisexualis*." Mycologia 32: 710-727. 1940.
23. RAPER, J. R.: "Sexual Hormones in *Achlya* II. Distance Reactions conclusive Evidence for a Hormonal Co-ordinating Mechanism." Amer. Jour. Bot. 27: 162-173. 1940.
24. RAPER, J. R.: "Sexual Hormones in *Achlya* III. Hormone A and the Initial Male Reaction." Amer. Jour. Bot. 29: 159-166. 1942.
25. RAPER, J. R. and HAAGEN-SMIT, A. J.: "Sexual Hormones in *Achlya* IV. Properties of Hormone A of *Achlya bisexualis*." Jour. Biol. Chem. 143: 311-320. 1942.
26. RAPER, J. R.: "Sexual Hormones in *Achlya* V. Hormone A, a Male Secreted Augmenter or Activator of Hormone A." Proc. Nation. Acad. Sci. U.S.A. 28: 509-516. 1942.
27. SERBINOW, I. L. K.: "Morfologie biologica grilov Pythiaceae. *Pythium perniciosum* nov. sp. parasit tabachnych sieiantsev." Scripta Bot. Horti Imp. 28: 1.
28. SMITH, R. L.: "Studies on two strains of *Aphanomyces laevis* found growing as Mould Parasites on Crayfish." Mycologia 32: 205-213. 1940.
29. SPARROW, F. K., Jr.: "Recent Contributions to our Knowledge of the Aquatic Phycomycetes." Biol. Rev. Cambridge Philos. Soc. 10: 152-186. 1935.
30. TROW, A. H.: "Observations on the Biology and Cytology of *Pythium ultimum* n. sp." Ann. Bot. 15: 269-312. 1901.
31. VANDENDRIES, R.: "La conduite sexuelle des Hymenomycetes interpretée par les theories des Hartmann concernant le bisexualite et la relativité sexuelle." Bull. Acad. Belg. V: 1213-1234. 1930.
32. WAGER, V. A.: "Descriptions of the South African Pythiaceae with Records of their Occurrence." Bothalia 4: 3-35. 1941.
33. WOLF, F. T. A.: "A Contribution to the Life History and Geographic Distribution of the genus *Allomyces*." Mycologia 33: 158-173. 1941.

II. EXPLANATION OF PLATES.

Figs. 1, 2, 4, 6, 8, 9 and 23 are drawn with the magnification 75 $\times$.

Figs. 3, 5, 7, 10-22, 24 and 25 are drawn with the magnification 250 $\times$.

- FIG. 1. *Saprolegnia delica*. Antheridia and oogonia.
- FIG. 2. *Saprolegnia delica*. (a) sporangium.
(b) chain of gemmae.
- FIG. 3. *Saprolegnia delica*. (a) young oogonium with monoclinal and declinal antheridia.
(b) antheridia and oogonia showing fertilisation tube approaching oospore.
(c) oogonium with mature oospores.
- FIG. 4. *Achlya imperfecta*. Antheridia and oogonia.
- FIG. 5. *Achlya imperfecta*. Oogonium with pits and one disorganising oospore.
- FIG. 6. *Achlya dubia*. Sporangia.
- FIG. 7. *Achlya dubia*. Oogonia with antheridia.
- FIG. 8. *Achlya dubia*. Chain of empty gemmae, antheridia and oogonia.
- FIG. 9. *Dictyuchus monosporus*. Antheridia and oogonia.
- FIG. 10. *Dictyuchus monosporus*. Antheridia and oogonia.
- FIG. 11. *Aphanomyces laevis*. (a) and (b) antheridia winding spirally round young oogonia.
(c) oogonium with mature oospore.
- FIG. 12. *Aphanomyces laevis*. (a) oogonium and coiling antheridial branches.
(b) antheridia, oogonium fertilisation tube.
- FIG. 13. *Aphanomyces stellatus*. (a) oogonium with declinal antheridium.
(b) oogonium with mature oospore.
(c) oogonium with monoclinal antheridium.
- FIG. 14. *Pythium dissotocum*. Sporangia.
- FIG. 15. *Pythium dissotocum*. (a) hypha with outgrowths.
(b) intercalary oogonium with declinal oospore.
- FIG. 16. *Pythium dissotocum*. (a) and (b) terminal oogonia with declinal antheridia.
(c) oogonium with one monoclinal and one declinal antheridium.
(d) oogonium with sessile antheridium.
- FIG. 17. *Pythium perniciosum*. (a) sporangium and irregular swollen region of hypha.
(b) sporangium and chain of asexual reproductive bodies.
- FIG. 18. *Pythium perniciosum*. (a) and (d) terminal oogonia with declinal antheridia.
(b) oogonium with two antheridia.
(c) young oogonium with 3 monoclinal antheridia.
- FIG. 19. *Pythium polytylum*. Sporangia.
- FIG. 20. *Pythium polytylum*. (a) lateral oogonium with declinal antheridium.
(b) intercalary oogonium with declinal antheridia.
(c) terminal oogonium with sessile antheridium.
(d) terminal oogonium with mature oospore.
- FIG. 21. *Pythium polytylum*. Oogonium with 1 monoclinal and 2 declinal antheridia.

- FIG. 22. *Pythium ultimum*. (a) terminal oogonium with monoclinal antheridium and mature oospore.
(b) terminal oogonium with monoclinal and diclinal antheridia.
(c) intercalary oogonium with monoclinal antheridium.
(d) intercalary oogonium with 2 monoclinal antheridia.
- FIG. 23. *Allomyces* sp. Sporangia with resting bodies.
- FIG. 24. *Allomyces* sp. Hyphae showing perforated pseudosepta.
- FIG. 25. *Allomyces* sp. (a) sporangium with escaping zoospores.
(b) chain of empty sporangia and resting body.

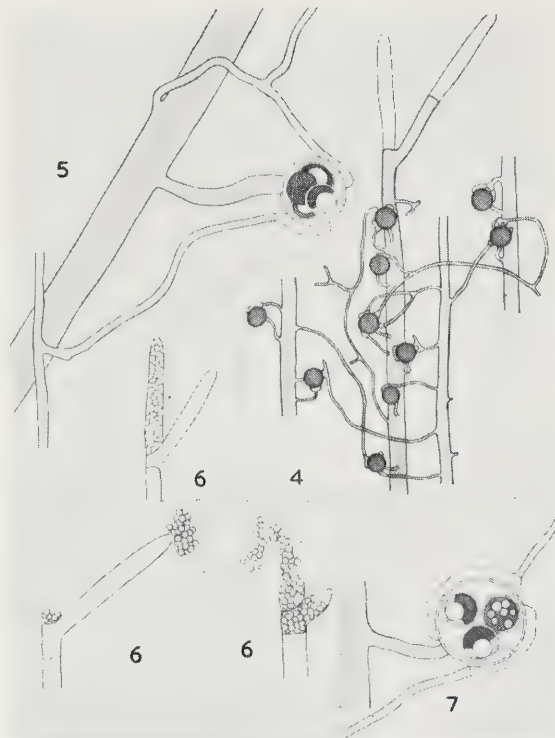
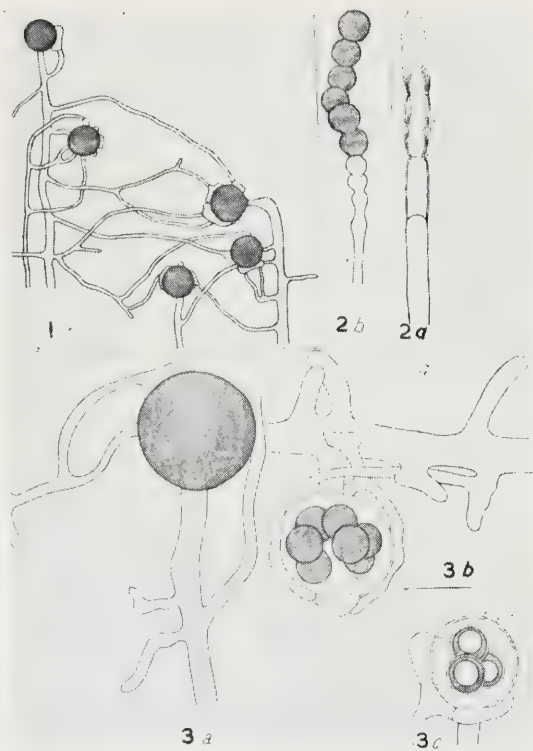


PLATE XV.

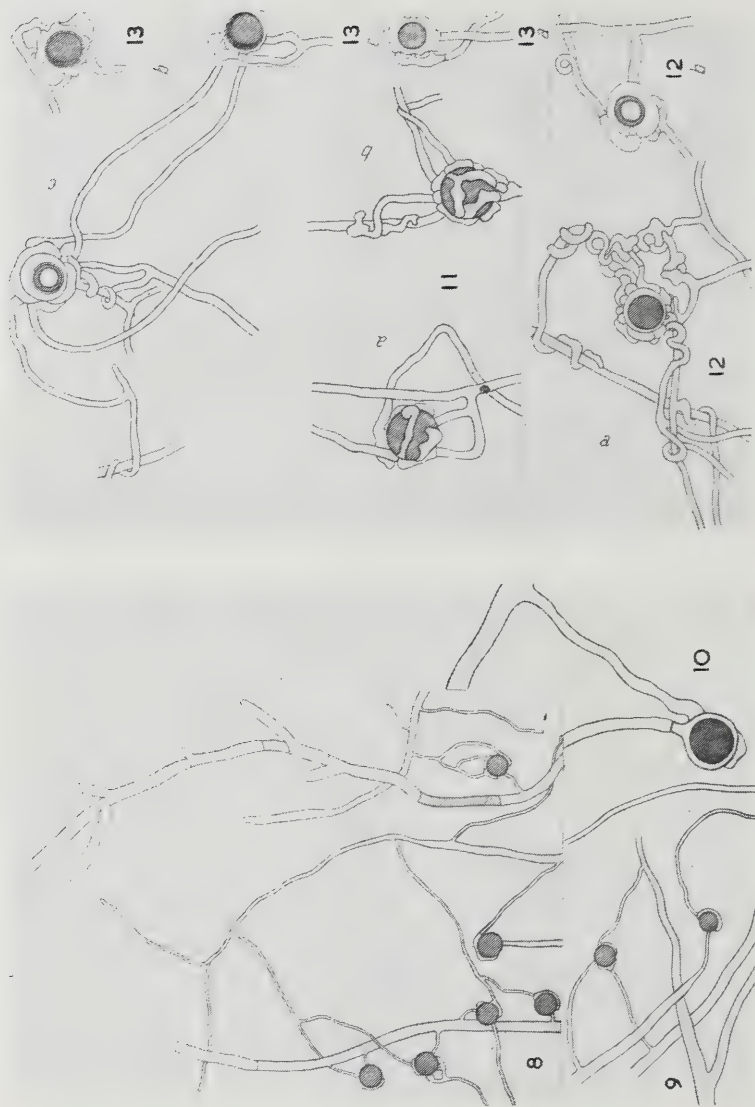


PLATE XVI.

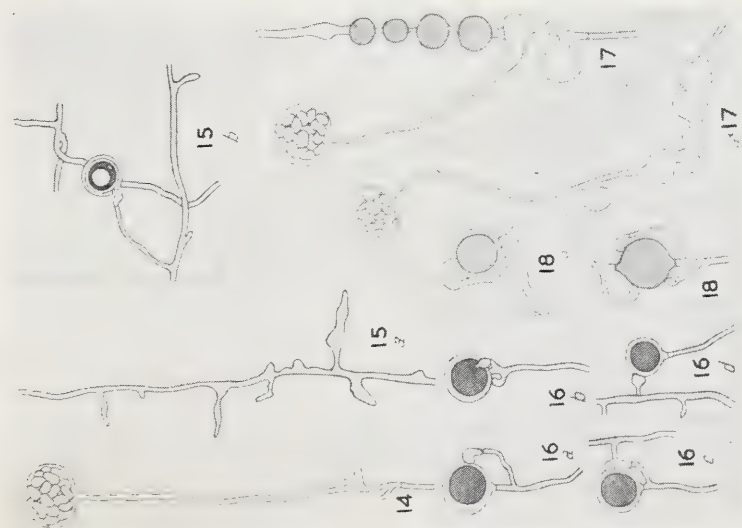
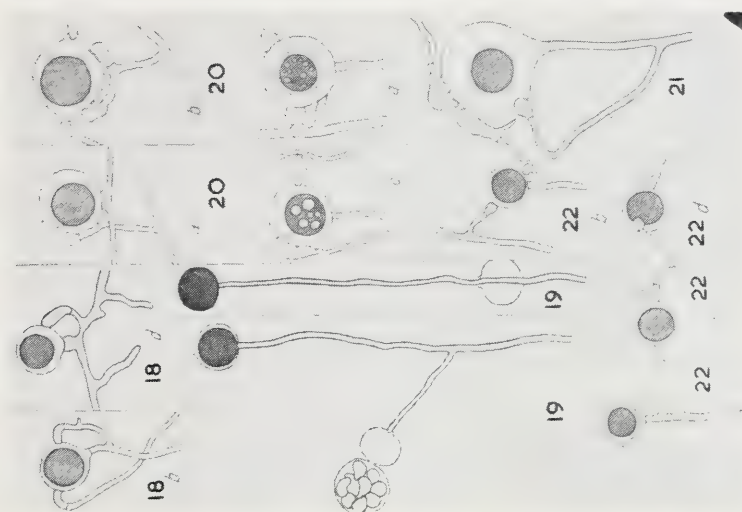
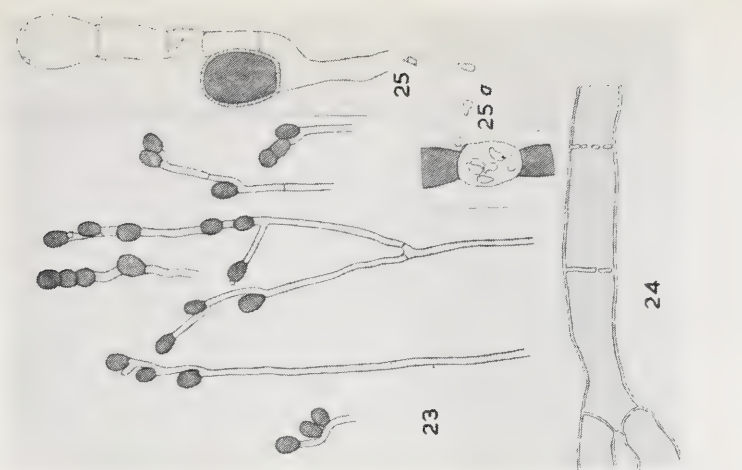


PLATE XVII.